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Comparative Growth Retarding Activity in Relation to Endogenous Tissue Concentration of Daminozide and a Pyrrolidino Analog (Uni-F529) in *Phaseolus vulgaris* L. and *Chrysanthemum morifolium* Ramat[1, 2]

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Abstract. N-pyrrolidino succinamic acid (Uni-F529) was considerably superior to succinic acid 2,2 dimethyl hydrazide (daminozide, SADH) in inhibiting stem elongation in *Phaseolus vulgaris* L. 'Black Valentine' and *Chrysanthemum morifolium* Ramat. 'Bright Golden Anne'. This was true in winter or summer greenhouses. Under controlled temperature and light conditions tissue concentrations of daminozide were higher than those for Uni-F529. Neither daminozide nor Uni-F529 was metabolized significantly in beans during the 3 to 7 day test periods. The greater activity of the pyrrolidino analog relative to daminozide must reflect increased activity at the site of action and/or reduced storage of Uni-F529 at inactive sites in plants.

Growth retardants have proven value for restricting excessive stem elongation without causing major phytotoxic side effects. Daminozide appears to offer great promise for growth control in many species, but it has a limited species range and, even where effective, it must be applied at relatively high concentrations and frequent intervals. For this reason there is a need for more active analogs. Five factors may account for differences in activity of foliar-applied compounds in the test plant: 1) absorption by the plant, 2) transport and distribution to sites of activity, 3) metabolic stability, 4) storage at inactive sites and 5) intrinsic biological activity. Several structure/activity studies have been made with other growth retardants but none has attempted to test all 5 components of activity. Rational development of improved growth retardants is dependent upon understanding which of the factors contributes to (or detracts from) the activity of promising compounds. Since Uni-F529 was reported to be more active than daminozide on several species (1, 2, 8) it was selected for comparison with SADH for studies on absorption, transport, and metabolic stability. Cathey (1) suggested that Uni-F529 was more active than daminozide under summer time greenhouse conditions only; hence, tests were made for activity relative to climatic conditions.

Materials and Methods

'Black Valentine' bean seedlings, *ca* 7 days old with the primary leaves half expanded and the first trifoliolate leaf beginning to unfold from the plumule, were used for all experiments. Seedlings were placed in aerated, half-strength Hoagland's in 1-liter containers or in greenhouse pots in vermiculite watered with half-strength Hoagland's. Temperature was maintained day and night at 21 ± 1°C; relative humidity 60 ± 10%. The daily photoperiod was 16 hours of 1300 to 2500 ft.c. light at plant top level provided by a mixture of "Cool White" flourescent tubes and incandescent lamps.

Rooted cuttings of 'Bright Golden Anne' chrysanthemum were grown under long day greenhouse conditions [8 hours natural light

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daily (*ca* 26°C) plus 4 hours of 10 ft.c. incandescent illumination from 10:00 p.m. to 2:00 a.m. provided under black cloth from 4:30 p.m. to 8:30 a.m (*ca* 19°C)]; when the plants had 3 fully expanded leaves they were treated with the retardants. Technical grade, crystalline retardants at least 95% pure were obtained from UniRoyal, Inc., Naugatuck, CN. Solutions contained 0.1% polyoxyethylene nonyl phenol surfactant.

When transport was studied the growth retardants were applied to the primary leaves by microsyringe in 8 to 10 droplets distributed above the 5 major veins. Plants were sprayed with 5×10^{-2} or 5×10^{-3} M solutions of each retardant for some comparative activity, absorption and metabolism studies.

There were 6 to 10 plants per treatment, randomized on benches. Stem length, the distance between the cotyledonary node and the plumule in bean, or between a marked leaf and the terminal bud in chrysanthemum, was measured initially and at the end of the experiment. Stem elongation (ΔL), the difference between the final and initial measurements was the sole measure of retardation recorded. Percent inhibition of elongation (Inh) was calculated for each treatment as: Inh = 100 (ΔL control − ΔL treatment/ΔL control) × 100. Additionally, the activity of Uni-F529 relative to daminozide was computed by dividing the value of percent inhibition for SADH by that for Uni-F529.

Chemical analysis of plant tissues. Prior to analysis the plant tissues were rinsed in running tap water; the tissues from 2 plants were pooled and frozen, or analyzed immediately after collection. Plant parts were ground to a puree in distilled water and washed into a one *L* reaction flask. The procedures for analysis of daminozide were essentially the same as those described by Lane (4) and Sachs and Mock (9) and extended to N-pyrrolidino succinamic acid. N-pyrrolidino succinamic acid yields N-amino pyrrole and daminozide yields 1,1 dimethylhydrazine upon alkaline hydrolysis, but colorimetric detection of both compounds was readily achieved with a 0.1% trisodium pentocyanomino ferroate (TPF) reagent. The absorption maximum for the 1,1 dimethylhydrazine-TPF complex is at 490 nm and that for the N-amino pyrrole-TPF complex is at 500 nm. In determining standard curves to minimize background interference from plant tissues the optical density (OD) difference used for Uni-F529 was OD_{500} OD_{600} and that for daminozide was OD_{490} OD_{600}. Bean and chrysanthemum shoot tissue gave background values equivalent to 0.05 micromoles per 7.5 g tissue. All data was corrected for background. The minimum tissue concentration of retardant detectable was approximately 0.01 micromoles per g fresh wt. Tests with daminozide added to bean tissue indicated that 94 ± 6% recovery could be expected with concentrations between 0.02 and 2 micromoles per g tissue. At least 2 determinations of tissue concentration were made for each treatment.

Concentration of retardant in the tissue (TC) was expressed as micromoles per g fresh tissue. Inh/TC was computed to give a measure of specific activity for each retardant that was independent of both dosage applied and amount absorbed.

Daminozide, ^{14}C-labeled at the 2 and 3 C atoms of the succinate moiety, 0.1 mc/milimole, and ^{3}H-Uni-F529, randomly labeled by a catalytic exchange procedure by New England Nuclear Corp. were used for comparative transport and metabolism studies. Both compounds were purified by paper and thin layer chromatographic procedures described below. Isotope counting was done with a liquid scintillation counter. Quench correction was determined by a channels ratio procedure using an external standard. Aliquots of methanolic extracts of tissues were counted directly or partitioned on silica gel (SG) thin layers or Whatman #1 paper. Two solvent systems were used: 1) 2-propanol (10) concnacetic acid (0.1) water (3); and 2) 1-propanol (7) concn NH_4OH (3). R_f values for SADH and Uni-F529 were:

	I (SG)	II (SG)	II (paper)
Daminozide	0.33	0.55	0.8
Uni-F529	0.47	0.62	0.75

Statistical analyses were performed on all data; either the least significant difference or Duncan's multiple range test at the 1% and 5% levels were used to separate means.

Results

Relative growth retarding activity. Daminozide and Uni-F529 at 5×10^{-2} M were sprayed on 'Black Valentine' bean in 7 experiments performed from December through August in the greenhouses at Frederick. Uni-F529 was always superior to SADH in growth retarding activity (Fig. 1), but their relative activity varied considerably and with no apparent relationship to the greenhouse environment. Relative activity of the 2 chemicals differed in 2 experiments run in August, overlapping by all but 1 day. Thus, time of year did not appear to influence the activity of one compound relative to the other. We could not, however, account for the highly variable results.

Similar patterns were obtained in 7-day experiments with 'Bright Golden Anne' chrysanthemum in the greenhouses at Davis (Fig. 2). Experiments were run in long day (LD) and short day (SD) environments. Uni-F529 was always superior to daminozide but, as with bean, relative activity was variable. Again, there was no apparent relationship to either time of year or to daylength. Light intensity and average daytime temperatures were considerably lower in November.

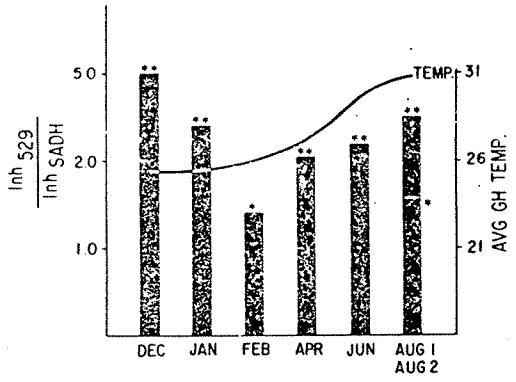

Fig. 1. Growth retarding activity of Uni-F529 relative to daminozide (SADH) in 'Black Valentine' bean at Frederick, MD. Plants were sprayed with 5×10^{-2} M solutions of each retardant. Average greenhouse temperatures for the 7-day experimental period for each experiment are connected by a line. The asterisks above the bars for relative activity indicate that the differences between inhibition induced by Uni-F529 and SADH were significant at the 1% (**) or 5% (*) level. Two experiments were run in August, overlapping by all but 1 day.

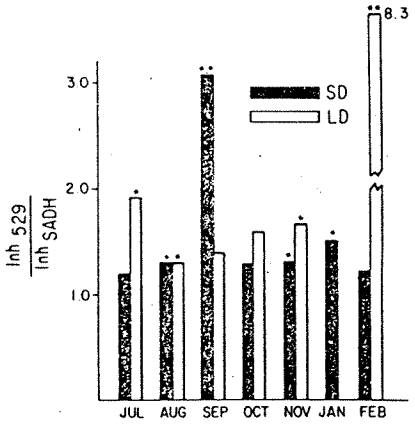

Fig. 2. Growth retarding activity of Uni-F529 relative to SADH in 'Bright Golden Anne' chrysanthemum in long day (LD) and short day (SD) conditions in greenhouses at Davis, CA. Plants were sprayed with 2.5×10^{-3} M solutions of each retardant. Asterisks above bars indicate statistical significance as in Fig. 1.

January, and February trials than those in July, August, and September.

Endogenous levels. Uni-F529 and daminozide levels were determined in the terminal 4 cm of shoot tissue of the chrysanthemum plants used to estimate relative growth retarding activity (Table 1). Inh/TC values for Uni-F529 were generally higher than those for daminozide but values for both were variable. Whole plant analyses for chrysanthemum (not reported) revealed that twice as much daminozide as Uni-F529 was absorbed but the concentrations in the terminal 4 cm of the shoots, the presumptive sites of action, were rarely significantly different. A few experments with 'Black Valentine' bean were performed in artificially lighted, controlled temperature (22°C) chambers to reduce as far as possible environmental variability as a contributing factor in absorption or response to retardants. A relationship occurred between the concn applied to primary leaves and endogenous levels in the tissues of the whole plant (Table 2), however, there was no apparent relationship between the amount of retardant applied and the degree of inhibition. The ratio, Inh/TC, which was computed to give some measure of intrinsic activity of the 2 compounds, dropped significantly when dosage was increased (Table 2) suggesting that much of the retardant absorbed by the leaves moved to sites that were inactive and had no effect on growth retardation. For the same dosage applied, about twice the concn of daminozide as of Uni-F529 was found in tissue and this was the major reason that Inh/TC was greater for Uni-F529 than for daminozide. Also, a comparison was made between foliar-applied and plumular-applied retardants (Table 3). One micromole of each retardant was applied to the plumular bud or primary leaf; the terminal 4 cm of shoot tissue only was analyzed for retardant content 3 days after application. Plumular applicatioas were much more effective than foliar for growth retardation. Here too, daminozide was absorbed more readily than Uni-F529. For plumular applications, the greater TC for SADH accounted for the relatively low computed value of Inh/TC.

The great dependency of Inh/TC on site of application suggests that much of the retardant found even in terminal meristematic tissues was not essential for growth retardation. Also, in bean, 75 to 80% Inh may have been the maximum attainable with either daminozide or Uni-F529.

Transport and distribution of Uni-F529. With application of ^{14}C-daminozide and ^{3}H-Uni-F529 relatively more of the radioactivity from ^{14}C-daminozide than from ^{3}H-Uni-F529 was found in the plumule above the treated leaf (Fig. 3) after 3 days but by 7 days the differences were reduced and not significant. Both compounds were translocated to the root system and then out into the soil medium. In time, a significant loss of growth retardant from bean plants would occur through the root system. Chromatographic analyses of extracts

Table 1. Growth retarding activity (Inh) of Uni-F529 and daminozide at different times of the year in relation to tissue concn (TC, micromoles per g tissue) in the terminal 4 cm of 'Bright Golden Anne' chrysanthemum shoot tissues. Plants grown in long day (LD) or short day (SD) conditions were sprayed with 2.5×10^{-3} M solutions of each retardant containing 0.1% polyoxyethylene nonyl phenol surfactant[z].

Time of treatment; Compound applied	LD			SD		
	Inh[y]	TC	Inh/TC	Inh	TC	Inh/TC
July						
Daminozide	25.1a	0.068a	368	30.9	0.059a	527
Uni-F529	42.1b	0.047b	905	35.2a	0.049a	712
August						
Daminozide	35.a	0.055a	633	35.6a	0.079a	448
Uni-F529	45.b	0.062a	727	46.2b	0.091a	509
September						
Daminozide	30.4a	0.049a	619	12.9a	0.049a	268
Uni-F529	43.2a	0.058a	751	37.4b	0.012b	3252
October						
Daminozide	24.9a	0.067a	373	32.4a	0.024a	1346
Uni-F529	38.4b	0.088a	437	42.1a	0.036a	1183
November						
Daminozide	25.8a	0.114a	226	26.6a	0.156a	170
Uni-F529	41.5b	0.099a	419	38.6b	0.155a	250
January						
Daminozide	—	0.105a	—	46.9a	0.104a	289
Uni-F529	—	0.034a	—	67.9b	0.041b	966
February						
Daminozide	3.3a	0.059a	55	30.2a	0.053a	885
Uni-F529	25.2b	0.068a	369	38.6a	0.071a	959

[z] Inhibition of growth retardant determinations were made 7 days after application.
[y] Pairs of means for each treatment date followed by different letters were significantly different at the 5% level.

Table 2. Activity and tissue concn of daminozide and Uni-F529 in 'Black Valentine' bean in relation to dosage applied[z].

Compound; Dosage	Inh[y]	TC	Inh/TC
Daminozide			
5 micromoles	34.5a	0.044b	785
10 micromoles	36.2a	0.149a	243
Uni-F529			
2.5 micromoles	36.3a	0.011d	3310
5.0 micromoles	41.3b	0.023c	1790
10.0 micromoles	40.3b	0.058b	695

[z] Measurements and analyses made 4 days after application of retardants to upper surface of both primary leaves. Analyses were for the entire plant. TC is tissue concn in micromoles per g tissue.
[y] Means in columns followed by different letters was significantly different at the 5% level.

Table 3. Activity of foliar and plumular applications of daminozide and Uni-F529 in 'Black Valentine' bean in relation to tissue concn of retardants in terminal shoot tissues.

Site of application, compound	Inh[y]	TC	Inh/TC
Primary leaves[z]			
Daminozide	10.6a[x]	0.021a	505
Uni-F529	43.2b	0.012b	3600
Plumule[y]			
Daminozide	76.1a	457a	16.7
Uni-F529	82.1a	199a	41.2

[z] 10 micromole of each retardant applied to upper surface of both primary leaves. Plumule plus 4 cm of subtending shoot tissue were analyzed for retardant concn 3 days after application. TC is tissue concn in micromoles per g tissue.
[y] 1 micromole of each retardant applied to plumule. Shoot tissue analyzed as above.
[x] Pairs of means, for each site of application, followed by different letters, were significantly different at 5% level.

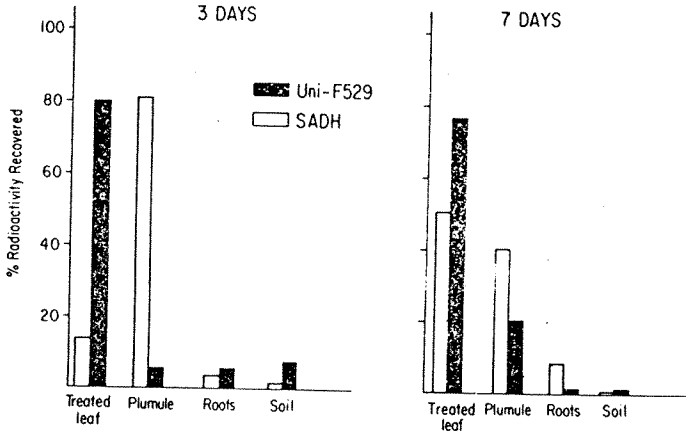

Fig. 3. Distribution of radioactivity in ^{14}C-daminozide (SADH) ^{3}H-Uni-F529 treated 'Black Valentine' bean plants 3 and 7 days after application. For each compound, ca 10^{-6} dpm, were applied to the first trifoliolate leaf of 6 plants. Plants were analyzed individually; average values are shown. Statistically significant differences between SADH and Uni-F529 were found only in the 3 day analyses for the plumule and treated leaf.

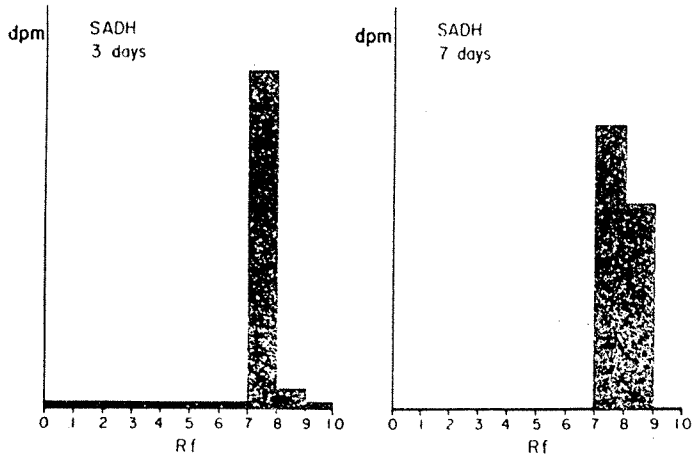

Fig. 4. Chromatographic fractionation of methanolic extracts of 'Bright Golden Anne' chrysanthemum shoot tissue from plants treated 3 days earlier with 1×10^{-2} M daminozide (SADH). Extracts were applied to SG thin layers and developed by ascending techniques in 2-propanol (10)/concn acetic acid (0.1)/water (3). Data are average values for 6 plants.

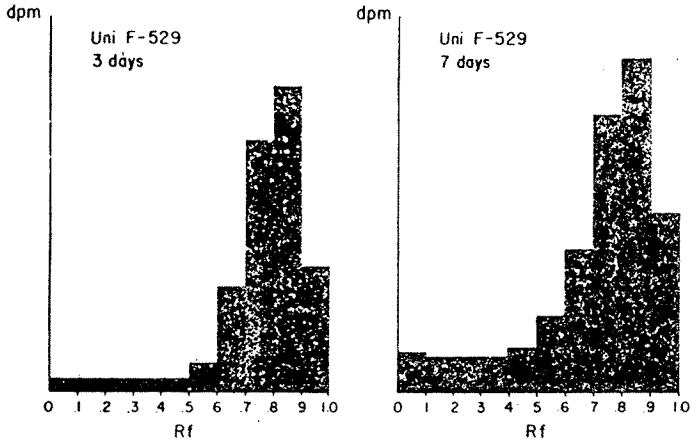

Fig. 5. Chromatographic fractionation of methanolic extracts of chrysanthemum shoot tissues from plants treated with Uni-F529. Extracts were applied to Whatman #1 paper and developed by descending chromatography in 1-propanol (7) NH_4OH (3). Data are average values for 6 plants.

of bean and chrysanthemum plants treated with ^{14}C-daminozide revealed no alteration of the compound up to 7 days after treatment (Fig. 4). Chromatographic analyses of Uni-F529-treated chrysanthemum plants suggest that after 7 days most of this compound remained unmetabolized (Fig. 5). Very little daminozide and Uni-F529, less than 0.01 micromoles per g, remained bound to tissues after either methanolic or aqueous extraction of chrysanthemum plants sprayed with 10^{-2} M solutions.

Discussion

The enhanced activity of the pyrrolidino analog, Uni-F529, compared to daminozide was not dependent upon time of year, an hypothesis proposed elsewhere (1, 2), nor due to any advantage in absorption by tissues. Distribution of Uni-F529 in bean and chrysanthemum, particularly in the shoot apical tissues, the presumptive sites of action, was approximately the same as that for daminozide. Daminozide proved to be a stable compound in bean and chrysanthemum, as has been shown in many species (5, 6, 7), and this was true for Uni-F529, too. Thus, we concluded that differences in growth retarding activity reflected greater activity of Uni-F529 than of daminozide in shoot apical and subapical meristematic tissues or less storage at inactive sites.

Storage of both compounds at inactive sites in chrysanthemum and bean meristematic tissues was suggested by decreasing values for inhibition per unit tissue concn (Inh/TC) of retardant with increased rates of application. That is, some of the compound found in meristematic tissues was probably not active in causing inhibition. Sachs and Mock (9) found this to be true also for 3 woody species. Inh/TC was always greater for Uni-F529 than for daminozide, but the values were not constant for either compound. Variable response of the treated plant to the retardant and/or variable storage of the compound at inactive sites owing to changes in meristematic tissues would account for changes in Inh/TC. We have noted, invariably, that the ratio Inh/TC increased with time after application as if the retardant at inactive sites were lost more rapidly than that at the active sites for inhibition.

Modification of Uni-F529 to enhance its absorption through the upper leaf surface or terminal bud to a level equal to that of daminozide would, perhaps, enhance 2- to 3-fold its activity in spray application. Sargent, Powell, and Blackman (10) have shown that increased chlorination of phenoxyacetic acids leads to an increase in the rate of absorption by bean leaves. Although there are certain exceptions to this rule, and notable variations in absorption of chlorinated compounds in light and darkness, their approach may be appropriate for Uni-F529.

In 'Black Valentine' bean plumular-applied daminozide and Uni-F529 was much more active, perhaps 20- to 30-fold, than foliar-applied chemicals. Thus, these compounds are transported in relatively small quantities from the leaves to the shoot apical and subapical meristems via the phloem, whereas absorption by the young leaves and meristematic surfaces supplied much larger quantities for the meristematic tissues. This was shown, also, in Dicks' studies with chrysanthemum (3) and implicit in the results of Sachs and Mock (9). There appeared to be very efficient inhibition of stem elongation by the small amounts translocated from the leaves; that is, the values of Inh/TC were higher for foliar than for plumular applications when only the TC for the terminal 4 cm of shoot tissues was used in the computation.

Literature Cited

1. Cathey, H. M. 1969. Enhancing activity of chemical growth retardants. *Southern Florist and Nurseryman*. pp. 18 20. Jan. 17.
2. ———. 1969. Selectivity in response to variation in the structure of succinic acid, 2,2-dimethylhydrazide (B995). *Plant Physiol.* 44(Suppl.): 24 25.
3. Dicks, J. W. 1972. Uptake and distribution of the growth retardant, aminozide, in relation to control of lateral shoot elongation in *Chrysanthemum morifolium*. *Ann. Appl. Biol.* 72:313 326.
4. Lane, J. R. 1968. General Alar residue method. Adaptation I and II. UniRoyal Chem. Co., Naugatuck, CN.
5. Martin, G. C., M. W. Williams, and L. P. Batjer. 1964. Movement and fate of labeled N-dimethyl amino succinamic acid (B-Nine), a size controlling compound in apple seedlings. *Proc. Amer. Soc. Hort. Sci.* 84:7 13.
6. Moore, T. C. 1968. Translocation of the growth retardant N,N-dimethyl amino succinamic acid—^{14}C - B-995-^{14}C). *Bot. Gaz.* 129:280 285.
7. Ryugo, K. and R. M. Sachs. 1969. *In vitro* and *in vivo* studies of Alar (1,1-dimethylamino succinamic acid, B-995) and related substances. *J. Amer. Soc. Hort. Sci.* 94:529-533.
8. Sachs, R. M., A. M. Kofranek, J. DeBie and J. Kubota. 1972. Reducing height of pot grown fuchsias. *Florist's Review* 150:85 87, 99 101.
9. ——— and T. Mock. 1975. Growth retarding activity of foliar applied daminozide (SADH) in relation to its concentration in three species. *J. Amer. Soc. Hort. Sci.* (in press).
10. Sargent, J. A., R. G. Powell, and G. E. Blackman. 1969. Studies on foliar penetration III. The effects of chlorination on the rate of penetration of phenoxyacetic acid and benzoic acid into leaves of *Phaseolus vulgaris*. *J. Expt. Bot.* 20:426 450.